10/009016
JC03 Rec'd PCT/PTO 04 DEC 2001

Practitioner's Docket No. U 013757-2

Optional Customer No. Bar Code

00140
PATENT TRADEMARK OFFICE

CHAPTER II

TRANSMITTAL LETTER TO THE UNITED STATES ELECTED OFFICE (EO/US) (ENTRY INTO U.S. NATIONAL PHASE UNDER CHAPTER II)

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/FI00/00220	17 MARCH 2000	24 JUNE 1999

TITLE OF INVENTION

PREVENTION OF TYPE 1 DIABETES AND OTHER NON-POLIO ENTEROVIRUS DISEASES

APPLICANT(S)

1. HEIKKI HYÖTY
2. MIKAEL KNIP

Box PCT
Assistant Commissioner for Patents
Washington D.C. 20231
ATTENTION: EO/US

NOTE: The completion of those filing requirements that can be made at a time later than 30 months from the priority date results from the Commissioner exercising his judgment under the authority granted under 35 USC 371(d). The filing receipt will show the actual date of receipt of the last item completing the entry into the national phase. See 37 C.F.R. §1.491 which states: "An international application enters the national state when the applicant has filed the documents and fees required by 35 USC 371(c) within the periods set forth in § 1.494 and § 1.495."

CERTIFICATION UNDER 37 C.F.R. 1.10*
*(Express Mail label number is **mandatory**.)*
(Express Mail certification is optional.)

I hereby certify that this correspondence and the documents referred to as attached therein are being deposited with the United States Postal Service on this date DECEMBER 4, 2001, in an envelope as "Express Mail Post Office to Addressee," Mailing Label Number EV 0110119507 US, addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

JENNIFER RASHKIN
(type or print name of person mailing paper)

Signature of person mailing paper

WARNING: Certificate of mailing (first class) or facsimile transmission procedures of 37 C.F.R. 1.8 cannot be used to obtain a date of mailing or transmission for this correspondence.

WARNING: Each paper or fee filed by "Express Mail" **must have the number of the "Express Mail" mailing label placed thereon prior to mailing. 37 C.F.R. 1.10(b).*
*"Since the filing of correspondence under § 1.10 without the Express Mail mailing label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will **not** be granted on petition." Notice of Oct. 24, 1996, 60 Fed. Reg. 56,439, at 56,442.*

EXPRESS MAIL LABEL NO.: EV 011019507 US

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WARNING: *Where the items are those which can be submitted to complete the entry of the international application into the national phase are subsequent to 30 months from the priority date the application is still considered to be in the international state and if mailing procedures are utilized to obtain a date the express mail procedure of 37 C.F.R. §1.10 must be used (since international application papers are not covered by an ordinary certificate of mailing - See 37 C.F.R. §1.8.*

NOTE: Documents and fees must be clearly identified as a submission to enter the national state under 35 USC 371 otherwise the submission will be considered as being made under 35 USC 111. 37 C.F.R. § 1.494(f).

1. Applicant herewith submits to the United States Elected Office (EO/US) the following items under 35 U.S.C. 371:

   a. [X] This express request to immediately begin national examination procedures (35 U.S.C. 371(f)).

   b. [X] The U.S. National Fee (35 U.S.C. 371(c)(1)) and other fees (37 C.F.R. § 1.492) as indicated below:

10009016.120402

2.Fees

CLAIMS FEE	(1) FOR	(2) NUMBER FILED	(3) NUMBER EXTRA	(4) RATE	(5) CALCULATIONS
[ ]*	TOTAL CLAIMS	35 - 20 =	15	x $ 18.00 =	$ 324.00
	INDEPENDENT CLAIMS	12 - 3 =	9	x $ 84.00 =	756.00
	MULTIPLE DEPENDENT CLAIM(S) (if applicable) + $280.00				
BASIC FEE**	[ ] U.S. PTO WAS INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY Where an International preliminary examination fee as set forth in § 1.482 has been paid on the international application to the U.S. PTO: [ ] and the international preliminary examination report states that the criteria of novelty, inventive step (non-obviousness) and industrial activity, as defined in PCT Article 33(2) to (4) have been satisfied for all the claims presented in the application entering the national stage (37 CFR 1.492(a)(4)) ..... $100.00 [ ] and the above requirements are not met (37 CFR 1.492(a)(1)) ..... $710.00 [X] U.S. PTO WAS NOT INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY Where no international preliminary examination fee as set forth in § 1.482 has been paid to the U.S. PTO, and payment of an international search fee as set forth in § 1.445(a)(2) to the U.S. PTO: [ ] has been paid (37 CFR 1.492(a)(2)) ..... $740.00 [ ] has not been paid (37 CFR 1.492(a)(3)) ..... $1,040.00 [X] where a search report on the international application has been prepared by the European Patent Office or the Japanese Patent Office (37 CFR 1.492(a)(5)) $890.00				
	Total of above Calculations				890.00
SMALL ENTITY	Reduction by ½ for filing by small entity, if applicable. Statement may also be filed. (note 37 CFR 1.9, 1.27, 1.28)				- 1,970.00
	Subtotal				985.00
	Total National Fee				$ 985.00
	Fee for recording the enclosed assignment document $40.00 (37 CFR 1.21(h)). (See Item 13 below). See attached "ASSIGNMENT COVER SHEET".				
TOTAL	Total Fees enclosed				$ 985.00

*See attached Preliminary Amendment Reducing the Number of Claims.

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i. [X] A check in the amount of $985.00 to cover the above fees is enclosed.
ii. [ ] Please charge Account No. _____ in the amount of $ _____.
A duplicate copy of this sheet is enclosed.

**WARNING: *"To avoid abandonment of the application the applicant shall furnish to the United States Patent and Trademark Office not later than the expiration of 30 months from the priority date: * * * (2) the basic national fee (see § 1.492(a)). The 30-month time limit may not be extended." 37 C.F.R. § 1.495(b).*

WARNING: *If the translation of the international application and/or the oath or declaration have not been submitted by the applicant within thirty (30) months from the priority date, such requirements may be met within a time period set by the Office. 37 C.F.R. § 1.495(b)(2). The payment of the surcharge set forth in § 1.492(e) is required as a condition for accepting the oath or declaration later than thirty (30) months after the priority date. The payment of the processing fee set forth in § 1.492(f) is required for acceptance of an English translation later than thirty (30) months after the priority date. Failure to comply with these requirements will result in abandonment of the application. The provisions of § 1.136 apply to the period which is set. Notice of Jan. 3, 1993, 1147 O.G. 29 to 40.*

3. [X] A copy of the International application as filed (35 U.S.C. 371(c)(2)):

NOTE: *Section 1.495 (b) was amended to require that the basic national fee and a copy of the international application must be filed with the Office by 30 months from the priority date to avoid abandonment "The International Bureau normally provides the copy of the international application to the Office in accordance with PCT Article 20. At the same time, the International Bureau notifies applicant of the communication to the Office. In accordance with PCT Rule 47.1, that notice shall be accepted by all designated offices as conclusive evidence that the communication has duly taken place. Thus, if the applicant desires to enter the national stage, the applicant normally need only check to be sure the notice from the International Bureau has been received and then pay the basic national fee by 30 months from the priority date." Notice of Jan. 7, 1993, 1147 O.G. 29 to 40, at 35-36. See item 14c below.*

a. [ ] is transmitted herewith.
b. [ ] is not required, as the application was filed with the United States Receiving Office.
c. [X] has been transmitted
i. [X] by the International Bureau.
Date of mailing of the application (from form PCT/IB/308): 4 JAN. 2001.
ii. [ ] by applicant on _____.
Date

4. [X] A translation of the International application into the English language (35 U.S.C. 371(c)(2)):
a. [X] is transmitted herewith.
b. [ ] is not required as the application was filed in English.
c. [ ] was previously transmitted by applicant on _____.
Date
d. [ ] will follow.

PCT/EP/060067

5. [X] Amendments to the claims of the International application under PCT Article 19 (35 U.S.C. 371(c)(3)):

NOTE: The Notice of January 7, 1993 points out that 37 C.F.R. § 1.495(a) was amended to clarify the existing and continuing practice that PCT Article 19 amendments must be submitted by 30 months from the priority date and this deadline may not be extended. The Notice further advises that: "The failure to do so will not result in loss of the subject matter of the PCT Article 19 amendments. Applicant may submit that subject matter in a preliminary amendment filed under section 1.121. In many cases, filing an amendment under section 1.121 is preferable since grammatical or idiomatic errors may be corrected." 1147 O.G. 29-40, at 36.

a. [ ] are transmitted herewith.
b. [ ] have been transmitted
   i. [ ] by the International Bureau.
   Date of mailing of the amendment (from form PCT/IB/308): _____.
   ii. [ ] by applicant on _____ (Date).
c. [X] have not been transmitted as
   i. [X] applicant chose not to make amendments under PCT Article 19.
   Date of mailing of Search Report (from form PCT/ISA/210): 25 AUG. 2000.
   ii. [ ] the time limit for the submission of amendments has not yet expired. The amendments or a statement that amendments have not been made will be transmitted before the expiration of the time limit under PCT Rule 46.1.

6. [X] A translation of the amendments to the claims under PCT Article 19 (38 U.S.C. 371(c)(3)):
a. [ ] is transmitted herewith.
b. [ ] is not required as the amendments were made in the English language.
c. [X] has not been transmitted for reasons indicated at point 5(c) above.

7. [X] A copy of the international examination report (PCT/IPEA/409)
[X] is transmitted herewith.
[ ] is not required as the application was filed with the United States Receiving Office.

8. [ ] Annex(es) to the international preliminary examination report
a. [ ] is/are transmitted herewith.
b. [ ] is/are not required as the application was filed with the United States Receiving Office.

9. [ ] A translation of the annexes to the international preliminary examination report
a. [ ] is transmitted herewith.
b. [ ] is not required as the annexes are in the English language.

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10. [X] An oath or declaration of the inventor (35 U.S.C. 371(c)(4)) complying with 35 U.S.C. 115
    a. [ ] was previously submitted by applicant on _____. Date
    b. [ ] is submitted herewith, and such oath or declaration
        i. [ ] is attached to the application.
        ii. [ ] identifies the application and any amendments under PCT Article 19 that were transmitted as stated in points 3(b) or 3(c) and 5(b); and states that they were reviewed by the inventor as required by 37 C.F.R. 1.70.
    c. [X] will follow.

Other document(s) or information included:

11. [X] An International Search Report (PCT/ISA/210) or Declaration under PCT Article 17(2)(a):
    a. [X] is transmitted herewith.
    b. [ ] has been transmitted by the International Bureau.
    Date of mailing (from form PCT/IB/308): _____.
    c. [ ] is not required, as the application was searched by the United States International Searching Authority.
    d. [ ] will be transmitted promptly upon request.
    e. [ ] has been submitted by applicant on _____. Date

12. [X] An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98:
    a. [X] is transmitted herewith.
    Also transmitted herewith is/are:
    [X] Form PTO-1449 (PTO/SB/08A and 08B).
    [X] Copies of citations listed.
    b. [ ] will be transmitted within THREE MONTHS of the date of submission of requirements under 35 U.S.C. 371(c).
    c. [ ] was previously submitted by applicant on _____. Date

13. [ ] An assignment document is transmitted herewith for recording.

A separate [ ] "COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" or [ ] FORM PTO 1595 is also attached.

_____
_____
_____

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14. [X] Additional documents:
    a. [X] Copy of request (PCT/RO/101)
    b. [X] International Publication No. WO 01/00236 A1
        i. [X] Specification, claims and drawing
        ii. [ ] Front page only
    c. [X] Preliminary amendment (37 C.F.R. § 1.121)
    d. [X] Other

FORM PCT/IB/308: FORM PCT/IB/332:
ONE (1) SHEET OF DRAWING (FORMAL)

15. [X] The above checked items are being transmitted
    a. [X] before 30 months from any claimed priority date.
    b. [ ] after 30 months.

16. [ ] Certain requirements under 35 U.S.C. 371 were previously submitted by the applicant on _____, namely:

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AUTHORIZATION TO CHARGE ADDITIONAL FEES

WARNING: *Accurately count claims, especially multiple dependent claims, to avoid unexpected high charges if extra claims are authorized.*

NOTE: "A written request may be submitted in an application that is an authorization to treat any concurrent or future reply, requiring a petition for an extension of time under this paragraph for its timely submission, as incorporating a petition for extension of time for the appropriate length of time. An authorization to charge all required fees, fees under § 1.17, or all required extension of time fees will be treated as a constructive petition for an extension of time in any concurrent or future reply requiring a petition for an extension of time under this paragraph for its timely submission. Submission of the fee set forth in § 1.17(a) will also be treated as a constructive petition for an extension of time in any concurrent reply requiring a petition for an extension of time under this paragraph for its timely submission." 37 C.F.R. § 1.136(a)(3).

NOTE: "Amounts of twenty-five dollars or less will not be returned unless specifically requested within a reasonable time, nor will the payer be notified of such amounts; amounts over twenty-five dollars may be returned by check or, if requested, by credit to a deposit account." 37 C.F.R. § 1.26(a).

[X] The Commissioner is hereby authorized to charge the following additional fees that may be required by this paper and during the entire pendency of this application to Account No. 12-0425 .

[X] 37 C.F.R. 1.492(a)(1), (2), (3), and (4) (filing fees)

WARNING: *Because failure to pay the national fee within 30 months without extension (37 C.F.R. § 1.495(b)(2)) results in abandonment of the application, it would be best to always check the above box.*

[ ] 37 C.F.R. 1.492(b), (c) and (d) (presentation of extra claims)

NOTE: Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must

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only be paid or these claims cancelled by amendment prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 C.F.R. § 1.492(d)), it might be best not to authorize the PTO to charge additional claim fees, except possible when dealing with amendments after final action.

[X] 37 C.F.R. 1.17 (application processing fees)
[X] 37 C.F.R. 1.17(a)(1)-(5)(extension fees pursuant to § 1.136(a).
[X] 37 C.F.R. 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 C.F.R. 1.311(b))

NOTE: Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 C.F.R. § 1.311(b).

NOTE: 37 C.F.R. 1.28(b) requires "Notification of any change in loss of entitlement to small entity status must be filed in the application . . . prior to paying, or at the time of paying . . . issue fee." From the wording of 37 C.F.R. § 1.28(b): (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity.

[ ] 37 C.F.R. § 1.492(e) and (f) (surcharge fees for filing the declaration and/or filing an English translation of an International Application later than 30 months after the priority date).

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SIGNATURE OF PRACTITIONER

Reg. No.: 25,858

WILLIAM R. EVANS
(type or print name of practitioner)

Tel. No.: (212)708-1930

LADAS & PARRY
P.O. Address

Customer No.: 00140

26 WEST 61ST STREET
NEW YORK, N.Y. 10023

#6

[illegible]'d PCT/PTO 12 MAR 2002

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: HEIKKI **HYÖTY,** ET AL.

Serial No.: 10/009,016 Group No.:

Filed: MARCH 12, 2002 Examiner:

For: PREVENTION OF TYPE 1 DIABETES AND OTHER NON-POLIO ENTEROVIRUS DISEASES

Attorney Docket No.: U013757-2

Assistant Commissioner for Patents
Washington, D.C. 20231

WRITTEN ASSERTION OF SMALL ENTITY STATUS

This is written assertion on the basis of:

☐ personal knowledge;

☐ applicant's letter of ______________;

☒ applicant's agent's letter of December 3, 2001; or

☐ other ______________

by a practitioner (not necessarily of record) that the above application is entitled to small entity status and, therefore, fees.

CERTIFICATION UNDER 37 C.F.R. 1.8(a) and 1.10*

(When using Express Mail, the Express Mail label number is mandatory; Express Mail certification is optional.)

I hereby certify that, on the date shown below, this correspondence is being:

MAILING

☒ deposited with the United States Postal Service in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

37 C.F.R. 1.8(a)

☐ with sufficient postage as first class mail.

37 C.F.R. 1.10*

☒ as "Express Mail Post Office to Address" Mailing Label No. EV011020981US (mandatory)

TRANSMISSION

☐ transmitted by facsimile to the Patent and Trademark Office.

Date: March 12, 2002

Jennifer L
Signature

JENNIFER RASHKIN
(type or print name of person certifying)

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"Since the filing of correspondence under § 1.10 without the Express Mail mailing label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will not be granted on petition." Notice of Oct. 24, 1996, 60 Fed. Reg. 56,439, at 56,442.

NOTE: *"To establish small entity status after the payment of the basic filing or national stage fee as a non-small entity, a written assertion of small entity status is required to be submitted." Notice of September 8, 2000, 65 Fed. Reg. 54604, at 54609.*

NOTE: *37 C.F.R. § 1.27(c)(1): "Assertion by writing. Small entity status may be established by a written assertion of entitlement to small entity status. A written assertion must:*

(i) Be clearly identifiable;

(ii) Be signed (see paragraph (c)(2) of this section); and

(iii) Convey the concept of entitlement to small entity status, such as by stating that applicant is a small entity, or that small entity status is entitled to be asserted for the application or patent. While no specific words or wording are required t assert small entity status, the intent to assert small entity status must be clearly indicated in order to comply with the assertion requirement."

NOTE: *37 C.F.R. § 1.27(c)(2): "Parties who can sign and file the written assertion. The written assertion can be signed by:*

(i) One of the parties identified in § 1.33.(b) (e.g. an attorney or agent registered with the Office). § 3.73(b) of this chapter notwithstanding, who can also file the written assertion;

(ii) At least one of the individuals identified as an inventor (even though a § 1.63 executed oath or declaration has not been submitted), notwithstanding § 1.33(b)(4), who can also file the written assertion pursuant to the exception under § 1.33(b) of this part; or

(iii) An assignee of an undivided part interest, notwithstanding §§ 1.33(b(3) and 3.73(b) of this chapter, but the partial assignee cannot file the assertion without resort to a party identified under § 1.33(b) of this part."

35 C.F.R. § 1.33(b):
(b) Amendment and other papers. Amendments and other papers, except for written assertions pursuant to § 1.27(c)(2)(ii) of this part, filed in the application must be signed by:

(1) A registered attorney or agent of record appointed in compliance with § 1.34(b);

(2) A registered attorney or agent not of record who acts in a representative capacity under the provisions of § 1.34(a);

(3) An assignee as provided for under § 3.71(b) of this chapter; or

(4) All of the applicants (§ 1.41(b)) for patent, unless there is an assignee of the entire interest and such assignee has taken action in the application in accordance with § 3.71 of this chapter.

Respectfully submitted,

CYNTHIA R. MILLER
C/O LADAS & PARRY
26 WEST 61ST STREET
NEW YORK, N. Y. 10023
REG. NO.: 34,678 (212)708-1914

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#4/a

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: HEIKKI HYÖTY & MIKAEL KNIP

International Application No.: PCT/FI00/00220

International Filing Date: 17 MARCH 2000

Priority Date Claimed: 24 JUNE 1999

For: PREVENTION OF TYPE 1 DIABETES AND OTHER NON-POLIO ENTEROVIRUS DISEASES

Attorney Docket No.: U 013757-2

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

PRELIMINARY AMENDMENT

Please amend the above-identified application as follows:

IN THE CLAIMS

3. (Amended) Use according to claim 1 for the manufacture of a vaccine to be administered in repeated doses to children.

6. (Amended) Use according to claim 1 for the manufacture of a vaccine to be administered to pregnant women to protect their offspring against said diseases.

CERTIFICATE UNDER 37 1.10

I hereby certify that this paper is being deposited with the United States Postal Service on this date DECEMBER 4, 2001 in an envelope as "EXPRESS MAIL POST OFFICE TO ADDRESSEE" Mailing Label Number EV 011019507 US addressed to the: Commissioner of Patents and Trademarks, Washington, D.C. 20231

JENNIFER RASHKIN
(Type or print name of person mailing paper)

(Signature of person mailing paper)

NOTE: Each paper or fee referred to as enclosed herein has the number of the "EXPRESS MAIL" mailing label place thereon prior to mailing 37 CFR 1.16(b).

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8. (Amended) Use according to claim 1 for the manufacture of a vaccine to be administered in combination with a vaccine, which induces serotype specific immunity against non-polio enteroviruses.

27. (Amended) The method of claim 19, wherein the administration of OPV is combined with the administration of a vaccine, which induces serotype specific immunity against non-polio enteroviruses.

Respectfully submitted,

WILLIAM R. EVANS
LADAS & PARRY
26 WEST 61ST STREET
NEW YORK, NEW YORK 10023
REG.NO.25,858(212)708-1930

MARKED-UP COPY

3. (Amended) Use according to claim 1 [or 2] for the manufacture of a vaccine to be administered in repeated doses to children.

6. (Amended) Use according to claim 1[or 2] for the manufacture of a vaccine to be administered to pregnant women to protect their offspring against said diseases.

8. (Amended) Use according to [any of] claim[s] 1 [to 7] for the manufacture of a vaccine to be administered in combination with a vaccine, which induces serotype specific immunity against non-polio enteroviruses.

27. (Amended) The method of [any of] claim[s] 19 [to 26], wherein the administration of OPV is combined with the administration of a vaccine, which induces serotype specific immunity against non-polio enteroviruses.

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Prevention of Type 1 diabetes and other non-polio enterovirus diseases

Field of the Invention

The invention relates to the prevention of Type 1 diabetes and other non-polio enterovirus diseases by a novel vaccination regime based on extensive immunisations by currently available oral poliovirus vaccine (OPV) and/or by new non-polio enterovirus vaccines.

The invention provides prevention of Type 1 diabetes mellitus (IDDM) and other non-polio enterovirus diseases by eliminating the risk effect of enterovirus infections. This is achieved by a novel immunisation regime, which is based on the induction of systemic and local mucosal Th1-type T-cell immunity by oral poliovirus vaccinations and optionally induction of Th2-type humoral immunity by a new enterovirus vaccine which induces neutralizing antibodies against appropriate enterovirus serotypes. These two regimes can be used separately or in combination.

More precisely the present invention relates to the use of oral poliovirus vaccine (OPV) for the manufacture of a vaccine against non-polio enterovirus diseases, and especially against Type 1 diabetes mellitus (IDDM). When OPV is used together with a vaccine, which induces serotype specific immunity against non-polio enteroviruses, harmful side effects of the non-polio enterovirus vaccine can be avoided. The invention thus provides a vaccine composition comprising said two vaccines.

Background

Enterovirus infections are usually subclinical but cause also various kind of diseases. Typical enterovirus diseases are meningitis, paralysis, myocarditis, generalized infections in newborns, hand, foot and mouth - disease, herpangina, pleurodynia, hepatitis, rash, exanthemas and respiratory diseases including pneumonia. In addition, enterovirus infections have been suspected to play a role in the pathogenesis of dilated cardiomyopathy, atherosclerosis, postviral fatique syndrome and Type 1 diabetes mellitus.

The group of enteroviruses includes a total of 64 different serotypes. Polioviruses are the most widely known enteroviruses including 3 different serotypes (poliovirus types 1, 2 and 3) which all can cause meningitis and typical paralytic poliomyelitis (flaccid paralysis). Meningitis is frequently caused by several non-polio enteroviruses, which are the most common cause of aseptic meningitis. Myocarditis is caused mainly by coxsackie B serotypes

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but also other enterovirus serotypes may be involved. Hand, foot and mouth - disease is mainly caused by certain coxsackie A serotypes and severe infections of infants are related to coxsackie B serotypes. Paralytic diseases can also be caused by some other serotypes than poliovirus serotypes. The serotypes related to atherosclerosis and Type 1 diabetes are not known. In type 1 diabetes the most suspected ones have been coxsackieviruses B4 and B5 but also other than coxsackie B serotypes may be involved.

The only enterovirus vaccine, which has been used in human beings is poliovirus vaccine. This vaccine includes all three poliovirus serotypes and gives effective prevention against paralytic poliomyelitis. The protection is based on the induction of neutralizing antibodies, against these serotypes and is serotype specific. Thus, neutralizing antibodies, which are induced by poliovirus vaccines do not protect against any other enterovirus serotypes than the three poliovirus serotypes. The role of T-cell mediated immune responses in the protection against poliovirus infections is not known. The generally accepted view is that they play only a minor role while antibodies are more important in the elimination of infection and in the protection against re-infections.

Two different types of poliovirus vaccine have been developed. The killed inactivated poliovirus vaccine (IPV; Salk vaccine) includes formalin-inactivated polioviruses (all 3 serotypes). This vaccine is given parenterally using subcutaneous injections. It induces a Th2-type immune response characterized by strong antibody response and high levels of neutralizing antibodies against all poliovirus serotypes and gives effective prevention against paralytic poliomyelitis. However, it induces only weak local immune response in the gut. As gut associated lymphoid tissue is the primary replication site of polioviruses, IPV vaccine can not protect against poliovirus infection but only against the complications of infections. IPV can induce only weak cytotoxic T-cell immune responses.

The other poliovirus vaccine is oral poliovirus vaccine (OPV; Sabin vaccine) which includes live attenuated polioviruses (all three serotypes). This vaccine is given *per os* and the virus replicates in the same way as the wild polioviruses in the body. As the vaccine is given *per os* in the same way as natural enterovirus infections are acquired, it induces strong local immunity in the intestine, which prevents from later poliovirus infections. Thus, OPV vaccinated individuals usually do not become infected by polioviruses because

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the virus is not able to replicate in the intestine. The nature of this protection is not completely understood but it probably depends on both neutralizing antibodies and T-cell mediated immunity. OPV induces stronger T-cell responses than IPV and it induces mainly Th1-type T-cell responses characterized by strong cytotoxic T-cell responses.

Vaccines against non-polio enteroviruses are not available for human use. The reason is that the large number of enterovirus serotypes makes it difficult to make a pan-enterovirus vaccine and, on the other hand, the serotypes, which are causing the most severe non-polio enterovirus diseases, are highly variable. Myocarditis and cardiomyopathies have been associated with coxsackie B group viruses, meningitis and neonatal infections with several different serotypes and practically nothing is known about the serotypes possibly related to the development of atherosclerosis. In Type 1 diabetes the responsible serotypes are not known except that polioviruses are not involved. The general view is that poliovirus vaccines should not be effective in the prevention of Type 1 diabetes or other non-polio enterovirus diseases, but that the prevention of non-polio enterovirus diseases would require new vaccines which should induce neutralizing antibodies against the serotypes to be protected. Another reason for the lack of human non-polio enterovirus vaccines is that the safety of such vaccines has not been reliably confirmed. Thus, there is no effective vaccine or any other treatment for the prevention of non-polio enterovirus diseases in man.

Inactivated and subunit vaccines which include certain coxsackie B viruses have been tested in animal models. They have induced good antibody levels in mice and rabbits and effectively protected from infections caused by the serotypes which were included in the vaccine (Fohlman et al., 1990 and 1993; See and Tilles, 1994 and 1997). However, these vaccines have not been tested in human beings. The main reason for this is that the current knowledge on the mechanisms of immune protection against enteroviruses is limited and the safety of such vaccines can not be guaranteed. The safety issue has become very important after the discovery of the unexpected side-effects related to the use of inactivated respiratory syncytial virus (RSV) and measles vaccines in humans (Fulginiti et al., 1967; Harris et al., 1969; Kapikian et al., 1969). These vaccines paradoxically increased the severity or modulated the course of natural infections. The most probable explanation for these adverse effects is that these kind of inactivated vaccines generally

induce good antibody response but very poor cytotoxic T-cell response. Thus, they may have induced a shift towards Th2-type antibody mediated immunity which resulted in the atypical symptoms. This indicates the need for very detailed data on the effect of the vaccine on the course of natural infections and careful evaluation of the safety issues.

Another problem has been that the protection which is achieved by vaccines of this kind depends on the induction of neutralizing antibodies and the protection is therefore serotype specific. Accordingly, the vaccine should include the serotypes, which should be prevented. As described above, in non-polio enterovirus diseases the spectrum of responsible serotypes varies a lot from disease to disease and even in one disease like Type 1 diabetes the exact serotypes of responsible viruses have not yet been identified. Thus, the composition of the enterovirus serotypes to be protected is not known and may be different from one disease to another.

The advantage of the immunisation regime of the present invention is that it is based on the oral poliovirus vaccine (OPV) which has been extensively used in almost all countries of the world and which has proved to be very safe and effective. The poliovirus vaccines are actually one of the most effective and safest vaccines ever developed and have led to an almost complete eradication of poliovirus infections from the world. The only clinically relevant complication of OPV is the risk of vaccine associated paralysis. However, its frequency is extermely low (about 1 per 1-10 milj. vaccinees).

The general view is that immunity against enterovirus infection is based on the presence of neutralizing antibodies against the virus. These antibodies can efficiently neutralize the virus when it enters the body. The significance of neutralizing antibodies is reflected by the fact that patients who have abnormally low levels of antibodies due to an immune deficiency are particularly susceptible for enterovirus infections. Neutralizing antibodies can be detected for prolonged periods after the infection. They contribute to the eradication of the virus during primary enterovirus infection and protect against reinfections. However, they can not protect against infections, which are caused by other serotypes. Thus, the protection by these antibodies is serotype specific. Accordingly, it is generally thought that it is essensial for the efficacy of enterovirus vaccines that the vaccine is able to induce high titres of neutralizing antibodies against the serotypes which should be protected. The

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only currently used enterovirus vaccine is poliovirus vaccine which includes all three poliovirus serotypes.

The present invention is based on the finding that, in contrast to the general paradigm, oral poliovirus vaccines could also protect against other enterovirus infections than poliovirus infections and could therefore be used for the prevention of various non-polio enterovirus diseases, which have been described in detail in previous paragraphs, and diseases where the role of enteroviruses has been suspected including Type 1 diabetes mellitus, chronic fatigue syndrome and atherosceloris. This protection would be based on efficient induction of T-cell responses and local mucosal immunity by repeated OPV vaccinations. T-cell immune responses are known to cross-react between certain enterovirus serotypes when analysed *in vitro* by T-cell proliferation assay (Beck and Tracy, 1990; Graham et al., 1993). However, it was not known whether this cross-reactivity had any biological significance *in vivo*. It was not either known to what extent T-cell responses which are induced by OPV vaccinations can cross-react with non-polio enteroviruses and whether this had any clinical relevance.

We have previously evaluated these questions by analysing enterovirus specific T-cell responses in young infants. We found that some infants, who had never experienced any coxsackievirus B infection according to the lack of neutralizing antibodies, had strong T-cell proliferation response against purified coxsackievirus B4 antigen, which probably reflects the cross-reactivity of T-cells which have initially been induced by other enterovirus infections (Juhela et al., 1998). In addition, polio vaccination at the age of 6 months induced stronger T-cell response to purified coxsackievirus B4 and poliovirus antigens in children who had serological evidence of previous enterovirus infection compared to children who had no previous enterovirus infections (Juhela et al., 1998). This suggests that T-cells can cross-react between polioviruses and non-polio enteroviruses.

Our aim is to utilise this T-cell cross-reactivity by priming cross-reactive T-cell memory using OPV vaccinations. This, in turn, would make the immune responses to other enteroviruses stronger and more rapid (secondary-type response) and in this way speed up the eradication of the virus during acute non-polio enterovirus infections. OPV can not totally protect from these infections as it does not induce neutralizing antibodies against non-polio enteroviruses but it may protect against viremia and severe illnesses by

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potentiating the T-cell responses by inducing cross-reactive memory T-cells. This kind of T-cell help can potentiate both the production of neutralizing antibodies during infection as well as cytotoxic T-cell responses against non-polio enteroviruses. It may also booster antibodies against other enteroviruses than the serotype causing the acute infection by eliciting anamnestic immune responses. Induction of anamnestic responses means that OPV stimulates memory T-cell clones, which have originated from previous enterovirus exposures and in this way leads to their activation and induction of antibodies against all these serotypes. This kind of anamnestic response is used in the present regime to enhance enterovirus antibody levels in pregnant women thus providing protection for their infants.

Summary of the Invention

One object of the present invention is to provide a method of preventing non-polio enterovirus diseases, especially Type I diabetes (IDDM).

Another object of the invention is to provide a vaccine or vaccine composition useful in preventing said diseases.

Still another object of the present invention is to avoid harmful side effects of killed or subunit enterovirus vaccines that induce serotype specific immunity.

Still another object of the present invention is the use of a polio vaccine and/or a non-polio enterovirus vaccine in the manufacture of a vaccine against enterovirus diseases, especially Type I diabetes (IDDM).

The objects of the present invention are fulfilled by providing a method of preventing non-polio enterovirus diseases or of preventing Type 1 diabetes mellitus (IDDM) comprising the administration of an effective amount of oral poliovirus vaccine (OPV) to a human subject.

The invention further encompasses the use of oral poliovirus vaccine (OPV) for the manufacture of a vaccine against non-polio enterovirus diseases, and especially for the manufacture of a vaccine against Type 1 diabetes mellitus (IDDM).

The invention is also directed to a vaccine composition comprising oral poliovirus vaccine (OPV) and a vaccine, which induces serotype specific immunity against non-polio enteroviruses. Preferably the non-polio enterovirus vaccine comprises enterovirus antigens representing diabetogenic enterovirus serotypes or a coctail thereof.

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The invention further relates to the use of a vaccine, which induces serotype specific immunity against one or more serotypes of diabetogenic non-polio enteroviruses selected from the group consisting of coxsackievirus B serotypes 1, 2, 3, 4, 5 and 6, echovirus serotypes 3, 4, 6, 9, 11, 22 and 30, and coxsackievirus A serotypes 9 and 16 for the manufacture of a vaccine against non-polio enterovirus diseases, especially Type 1 diabetes mellitus (IDDM). It also relates to said vaccine and to a method of preventing non-polio enterovirus diseases, especially IDDM, comprising administering an effective amount of said vaccine to a human subject.

The invention further provides a method of preventing non-polio enterovirus diseases, especially Type I diabetes mellitus (IDDM) in the offspring comprising the administration of an effective amount of oral poliovirus vaccine (OPV) to pregnant women, or comprising the administration of an effective amount of oral poliovirus vaccine (OPV) prenatally to the pregnant woman and postnatally to the baby.

A method of preventing non-polio enterovirus diseases, especially IDDM, comprising the administration of repeated doses of an effective amount of oral poliovirus vaccine (OPV) to children is provided.

Finally the invention encompasses a method of avoiding harmful side effects of non-polio enterovirus vaccines, which induce serotype specific immunity against non-polio enteroviruses comprising administering an effective amount of said non-polio enterovirus vaccine simultaneously, before or after administering an effective amount of oral poliovirus vaccine (OPV) to a human subject.

Brief Description of the Drawing

Figure 1 shows the cumulative prevalence of IDDM in cohorts which have never received OPV, or which have received one dose of OPV in childhood or *in utero*.

Detailed Description of the Invention

"OPV" is an abbreviation of oral poliovirus vaccine, and means a vaccine that comprises live attenuated polioviruses of one, two or all three of the serotypes or infectious cDNA or RNA thereof. Besides being administered orally OPV may also be given by any other mucosal route like *per rectum* or intranasally or it may be given parenterally. OPV comprising attenuated viruses of all three serotypes is commercially available and is also called Sabin vaccine.

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"IPV" is another commercially available poliovaccine, which comprises killed inactivated polioviruses of all three serotypes. This vaccine is called Salk vaccine.

"IDDM" means insulin-dependent diabetes mellitus, which is the same as Type 1 Diabetes Mellitus or Type 1 Diabetes.

"Non-polio enterovirus diseases" means any disease caused by non-polio enteroviruses e.g. meningitis, paralysis, myocarditis, generalized infections in newborns, hand, foot and mouth -disease, herpangina, pleurodynia, hepatitis, rash, exanthemas, respiratory diseases including pneumonia, dilated cardiomyopathy, atherosclerosis, postviral fatigue syndrome and Type 1 diabetes mellitus.

"An effective amount" of a vaccine is an amount, which is able to elicit a protective immune response in the recipient, either by eliciting neutralizing antibodies or a cell-mediated response, or both.

A vaccine that induces "serotype specific immunity" is the same as a vaccine that induces neutralizing antibodies. Such vaccines may be killed vaccines, subunit vaccines or cDNA or RNA fragment vaccines, wherein the fragment encodes an antigenic part or an inactivated form of the virus.

A "killed vaccine" is the same as an inactivated vaccine i.e. a vaccine comprising viruses treated so that they have lost their infectivity. A "subunit vaccine" comprises only an antigenic part or parts of the viruses, not the whole viruses.

A "diabetogenic enterovirus" is an enterovirus that is associated with the induction of diabetes. These viruses are represented by the group consisting of coxsackievirus B serotypes 1, 2, 3, 4, 5 and 6, echovirus serotypes 3, 4, 6, 9, 11, 22 and 30, and coxsackievirus A serotypes 9 and 16. However, also other serotypes of non-polio enteroviruses might be involved in the induction of diabetes.

Each of all three poliovirus serotypes can cause paralytic disease. Oral poliovirus vaccine used in the present invention may contain only one of them or their different combinations. Preferably it contains a cocktail of all these three serotypes (serotypes 1-3). The vaccine viruses of the OPV used are attenuated polioviruses, the virulence of which has been reduced. This may be carried out by different methods including serial passage of the virus in cell cultures, antigenic modification by chemical treatments, construction of recombinant or chimeric viruses, mutagenization of viral genome, deletion of

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certain gene regions, selection of temperature sensitive mutants or irradiation. Alternatively, vaccine viruses may be attenuated natural poliovirus isolates or infectious poliovirus cDNA or RNA having reduced capability to cause clinical disease. Typically, the presented immunisation regime is based on the use of the commercially available and widely used Sabin oral poliovirus vaccine, which contains all three poliovirus serotypes in each vaccine dose. It is administered orally and replicates in the intestine, but does not cause paralytic polio or other clinical manifestations.

Each immunising dose of OPV includes infective viruses or infective RNA or cDNA in a titre, which is able to produce infection in humans. This dose would correspond to that which is used in the traditional Sabin-type live oral poliovirus vaccine including a minimum of $10^{5.5}$ - 10^{6} $TCID_{50}$ for poliovirus Type 1, 10^{5} $TCID_{50}$ for poliovirus type 2 and $10^{5.5}$ - $10^{5.8}$ $TCID_{50}$ for poliovirus type 3 live attenuated Sabin strains of polioviruses. The dose may also be another, if it has been confirmed to be safe and infectious. (TCID = tissue culture infectious dose; $TCID_{50}$= the dose which infects 50 % of the cultures.)

The new non-polio enterovirus vaccines of the present immunisation regime may include either whole viruses, the infectivity of which has been inactivated, or sub-unit vaccines containing certain antigenic structures of the virus, or their combination, or fragments of viral RNA or cDNA coding for antigenic structures of the virus. Inactivated vaccines may be produced by propagating the virus in cell cultures and by purifying it from infected cells and culture media by high-speed centrifugation in a density gradient formed by sucrose or other high-density media. Alternatively the virus could be purified by chromatography. The infectivity of the purified viruses is destroyed by inactivating the viruses by chemical treatment (e.g. formalin inactivation like that used to produce IPV), irradiation or heat treatment. Sub-unit vaccines may consist of purified viral proteins or recombinant viral proteins, synthetic peptides corresponding to viral antigenic epitopes or empty viral capsids, which are produced during infection but lack the viral genome. These subunit vaccines can be administered either as such or conjugated to haptens or carriers (e.g. ISCOM particles).

The new non-polio enterovirus vaccines can be given parenterally or by mucosal route like *per os*, *per rectum* or intranasally. Each immunising dose includes viral structures in a titre, which is able to induce proper immune response in humans. This dose would correspond to that used in Salk-type

inactivated poliovirus vaccine including 1.8 - 2 μg of viral protein per each dose and 20 - 40 antigenic D-units of poliovirus type 1, 4 - 8 antigenic D-units of poliovirus type 2 and 16 - 32 antigenic D-units of poliovirus type 3. The dose may also be another, if it has been confirmed to be safe and immunogenic.

In addition to the active ingredients that elicit an immune response, the OPV and the non-polio enterovirus vaccines used in the present invention may comprise pharmaceutically acceptable excipients, carriers, haptens and adjuvants. Excipients, carriers, haptens and adjuvants may include for example phenoxyethanol, magnesium chloride, sucrose, thiomersal, formaldehyde, phenol, antibiotics (preservatives) or aluminium salts, ISCOM particles, carrier proteins (e.g. cholera toxin), liposomes or protein micelles (haptens/adjuvants).

A new immunisation regime for the prevention of diseases caused by non-polio enteroviruses is introduced (Table 1).

Table 1. Main immunization regime

Action 1. *OPV during pregnancy*
Given preferentially during the first trimester but may also be given later during pregnancy. May also be given to women who are at fertile age but not pregnant.

Action 2. *OPV in childhood*
Given at the age of 0, 6, 10, 14 weeks, boosters at older age (e.g. every 5 years).

Action 3. *Killed/subunit vaccine*
Given at the age of 3, 6 and 12 months, boosters at older age. Can also be given to pregnant mothers.

Actions 1, 2 and 3 can be used separately or in different combinations. The timing of childhood OPV vaccinations in action 2 may vary but the first ones should preferentially be given by the age of 3 months.

Killed or subunit vaccine includes one or more of the following enterovirus serotypes or their antigenic structures: coxsackievirus B serotypes 1, 2, 3, 4, 5 and 6, echovirus serotypes 3, 4, 6, 9, 11, 22 and 30, coxsackievirus A serotypes 9 and 16. It can be given during pregnancy and at varying ages in childhood with booster given later in life. Killed or subunit enterovirus vaccine may be given simultaneously, before or after OPV is given.

Protection against non-polio enteroviruses is induced by extensive immunisation by repeated doses of live attenuated oral poliovirus vaccine (OPV). The regime includes prenatal and postnatal OPV vaccinations, which can be used in combination or separately. Prenatal vaccination is carried out by giving OPV to pregnant women in order to protect the child *in utero* and in infancy (Action 1 in Table 1). This protection is based on anamnestic immune

response, which is induced by OPV-vaccination. Anamnestic response is due to the activation of cross-reactive enterovirus specific memory T-cell clones and leads to increases in antibody levels against those enterovirus serotypes to which the mother has been exposed prior to the OPV vaccination. Anamnestic antibody response of the mother protects the child because IgG class maternal antibodies are transferred to the fetus through the placenta and are thus protecting the child until the age of 6-12 months when maternal antibodies disappear from child´s circulation.

Postnatal vaccination (Action 2) is carried out like OPV vaccination shedules in general but may be more extensive to get maximal stimulation of cross-reactive T-cell immunity (Table 1). It includes repeated vaccinations, first ones given at birth and during the first weeks of life followed by booster vaccinations in childhood with a few years intervals (like in WHO EPI-program). OPV-vaccination *per os* induces also strong local immune response in mucosal surfaces, particularly in the gut. This is important because the primary replication site of enteroviruses is gut-associated lymphoid tissue. This local immunity is targeted also to non-polio enteroviruses because of OPV induced cross-reactive T-cell response and induction of local production of interferons.

The Actions 1 and 2 of this regime can be combined with new non-polio enterovirus vaccines, which induce serotype specific immunity to get maximal protective effect against non-polio enteroviruses (Action 3 in Table 1). Serotype specific immunity may be induced by killed enterovirus particles or sub-unit vaccines carrying certain enterovirus structures or peptides. This serotype specific vaccine can be given to pregnant mothers as well as to children as described in Table 1. The serotype specific vaccine preferably includes one or more of the following enterovirus serotypes (coxsackievirus B serotypes 1, 2, 3, 4, 5 and 6, echovirus serotypes 3, 4, 6, 9, 11, 22 and 30, and coxsackievirus A serotypes 9 and 16). This kind of killed or subunit vaccines induce efficient antibody response but the protection is specific for those viruses which are included in the vaccine (protection by neutralizing antibodies is serotype specific). In such combination OPV can be used to give additional protection by cross-reactive T-cell responses against the serotypes which are not included in the killed/subunit vaccine. OPV can also be used to booster the antibody responses which are induced by killed/subunit vaccines. OPV can also be used to direct the immune responses induced by

killed/subunit vaccines to Th1-type responses rather than Th2-type responses. Th2-type responses are typically induced by killed/subunit vaccines and can be associated with serious side-effects leading to more severe course of natural infection in vaccinated individuals (like observed in individuals vaccinated by killed RSV or measles vaccines). OPV like other live vaccines induces mainly Th1-type responses leading to cytotoxic T-cell responses and can thus counteract the Th2-type responses induced by killed/subunit vaccines by inducing cross-reactive Th1 -type T-cells. To avoid Th2-responses OPV may be given either before, simultaneously with or after the killed/subunit vaccines are given.

Thus, the present regime includes OPV-vaccinations to induce systemic T-cell responses and local mucosal immunity as well as anamnestic antibody responses in pregnant mothers (Actions 1 and 2). OPV vaccinations can be combined with new inactivated or subunit enterovirus vaccines (Action 3). This combination would give maximal preventive effect (neutralizing antibodies induced by killed/ subunit vaccines are the first barriers against infections and T-cell immunity induced by OPV helps in the eradication of infection). OPV may also be used in combination with inactivated or subunit vaccines to prime or booster their effect or to prevent possible harmful side-effects caused by Th2-type bias in immune response to enteroviruses which may be caused by inactivated or subunit vaccines.

We have found that there are unexpected side-effects of IPV vaccines, which increase the risk of complications of non-polio enterovirus infections like Type 1 diabetes by directing the immune response against non-polio enteroviruses into the Th2 direction. However, OPV is benefical, because it decreases the risk of complications of non-polio enterovirus infections and vaccinations of inactivated/subunit non-polio enterovirus vaccines (e.g against diabetes) by inducing cross-reactive memory T-cells, by directing the immune response to non-polio enteroviruses into the Th1 direction and by inducing local protection in the mucosal tissues.

One advantage of this invention is that it is based on a widely used and very safe vaccine (OPV) but gives a new indication for this vaccine, which has not been previously suggested. The novel aspects are also that the invention utilises strong T-cell responses induced by live OPV vaccine, the cross-reactivity of these responses between different enterovirus serotypes, induction of local immune responses by OPV in mucosal surfaces in pharynx

and in gut, vaccination of both pregnant women and children, and optional combination of OPV and new serotype specific killed/subunit vaccines to booster their effect and to avoid their side-effects related to Th2-based responses. An additional novel aspect is that the inactivated/subunit vaccine includes serotypes, which are the most important in the pathogenesis of severe non-polio enterovirus diseases including Type 1 diabetes.

This vaccination regime can be used in the whole population or in specific high-risk groups such as children with genetic risk alleles for Type 1 diabetes, children with diabetes in first-degree relatives or children positive for diabetes-related autoantibodies.

This vaccination regime is the only possibility which is currently available for the prevention of non-polio enterovirus diseases in man. It can be implemented into clinical work immediately as it is based on currently widely used and well-tolerated vaccine (OPV). It can be coupled with inactivated or subunit enterovirus vaccines to increase their preventive effect and to avoid their side-effects.

In Finland practically the whole population was vaccinated by one dose of OPV in February - March in the year 1985 to eradicate the last polioepidemic (Hovi et al., 1986; Harjulehto-Mervaala et al., 1994). This provides an excellent possibility to analyze possible effects of OPV vaccination on the risk of type 1 diabetes because IPV has been used as the only poliovirus vaccine for decades and has also been used after the epidemic. OPV vaccination was also given to pregnant women (Harjulehto-Mervaala et al., 1993). We have analysed the cumulative prevalence of type 1 diabetes in birth cohorts which have received OPV vaccination in the year 1985 either in childhood or *in utero* and compared that to cumulative prevalence in birth cohorts who had never received OPV (Figure 1). The Figure shows the cumulative prevalence of Type 1 diabetes (IDDM) per 100,000 children by the age of 8 years in Finland in birth cohorts which have either never received oral poliovirus vaccine (OPV) or have been vaccinated by one dose of OPV in childhood or *in utero* during the mass-vaccination campaign in 1985. The cumulative prevalence of type 1 diabetes was significantly lower in OPV-vaccinated cohorts compared to unvaccinated cohorts: The average prevalence in OPV vaccinated cohorts born in the years 1980-1985 was 272 compared to 326 in unvaccinated cohorts born in 1986-1989 ($p<0.01$ in student´s t-test). The prevalence of diabetes was also low in children whose

mother had been vaccinated during pregnancy (261 per 100,000; Figure 1). These findings indicate that both Actions 1 and 2 in the proposed immunisation regime (see Table 1) have a protective effect against Type 1 diabetes.

We have also found that incidence of Type 1 diabetes correlates with the type of poliovirus vaccine used in different countries. This correlation is not absolute but there is a general tendency to a lower incidence of Type 1 diabetes in countries where OPV is used compared to countries where inactivated (killed) poliovirus vaccine (IPV) is used. In Finland the incidence of Type 1 diabetes is the highest in the world, and Finland is also one of the very few countries where IPV has been used as the only poliovirus vaccine for several decades (except in the year 1985 as mentioned before).

A possible role of different poliovaccination regimes as a cause of the international differences in the incidence of Type 1 diabetes is also supported by our findings in Estonian and Finnish children. In Estonia, where the incidence of Type 1 diabetes is one third of that in the neighbouring Finland, OPV is used as the only poliovirus vaccine in contrast to Finland where IPV is used. We analysed T-cell proliferation responses to tetanus toxoid, poliovirus type 1, coxsackievirus B4 (CBV4) and adenovirus antigens in 9-months-old infants in both countries. The responses to poliovirus and CBV4 were significantly higher in Estonian than in Finnish children ($p<0.05$) while responses to other antigens did not differ between the groups. Neutralizing antibodies against CBV group enteroviruses did not differ between the groups suggesting that the observed difference in T-cell responses was not due to different exposure of infants to enteroviruses in the two countries. Accordingly, the higher T-cell response to purified CBV4 virus in Estonian children probably reflects cross-reactivity of T-cells primed by previous OPV vaccinations. In Finland, the IPV vaccine is used which does not induce as high T-cell responses as OPV and which is also given at older age than OPV in Estonia (Estonian children had received three doses of OPV compared to one dose of IPV in Finnish children by the age of 9 months). This suggests that the OPV vaccination schedule in Estonia induces stronger cross-reactive immune response to non-polio enteroviruses than the IPV vaccination schedule used in Finland. This indicates that Action 2 in our immunisation regime (see Table 1) has a protective effect against Type 1 diabetes.

In the Finnish Diabetes Prediction and Prevention study (DIPP) we have analysed the frequency and serotype of enterovirus infections in 21 infants who have been followed from the birth and who have manifested with clinical Type 1 diabetes or turned positive for diabetes-related autoantibodies as a marker of subclinical beta-cell damage. Enterovirus infections were more frequent in these children than in 104 control children matched for the time of birth, gender and HLA-risk alleles for Type 1 diabetes ($p<0.03$). This difference was particularly clear in infections which occurred 0-6 months before autoantibodies appeared: 57% of autoantibody positive subjects had an enterovirus infection during that period compared to 31% of control subjects of the same age (OR 3.7, 95% CI 1.2-11.4) (unpublished observation). During this period 29% of autoantibody positive children were positive for enterovirus RNA in serum compared to 6% of control subjects (OR 8.4, 95% CI 1.7-40.2). The results suggest that enterovirus infections are important risk factors for Type 1 diabetes and able to initiate the beta-cell damaging process in genetically susceptible individuals. The average age of the infants at the appearance of autoantibodies was 9 months suggesting that diabetogenic enterovirus infections may occur already during the very first months of life.

The serotype of enterovirus infections related to induction of autoantibodies or manifestation of clinical diabetes has been analysed in the DIPP study and in the previous Childhood Diabetes in Finland (DiMe) study. These serotypes are included in the killed/subunit vaccine in the present immunisation regime (Action 3 in Table 1).

OPV vaccinations can be combined not only with serotype specific vaccines but also with passive immunisation regimes against enteroviruses. This kind of passive immunisation may include e.g. immunoglobulins which contain enterovirus specific antibodies and which are given intravenously or orally.

Example 1

We have analysed the effect of OPV vaccination on the course of subsequent coxsackievirus B3 (CBV3) infection in mice. In these studies we used a transgenic BALB/c strain which expresses human poliovirus receptor and can therefore be infected by human polioviruses (Horie et al., 1994).

Transgenic BALB/c mice were first immunized by live poliovirus vaccine (Sabin strain of poliovirus type 1) or inactivated poliovirus vaccine IPV, and later challenged to a pancreas-tropic strain of coxsackievirus B3 (Nancy

strain). (IPV was the commercially available poliovirus vaccine Novum purchased by National Public Health Institute of Holland). Two doses of live poliovirus vaccine strain type 1 (Sabin) were given intramuscularly with two weeks intervals (10^6 $TCID_{50}$/mouse, first injection at the age of 8 weeks). Two doses of killed poliovirus vaccine were given intramuscularly in the same way (0.1 μg per mouse). Two weeks after the last poliovirus injection the mice were infected by coxsackievirus B3. T-cell proliferation responses were analysed two weeks after the coxsackievirus B3 challenge using standard blast-transformation test and highly purified viruses as antigens. The T-cell responses are expressed as spesific counts (mean cpm), and and the results are shown in Table 2.

Table 2. Effect of previous poliovirus immunisation on T-cell proliferation responses during subsequent coxsackievirus B3 infection in transgenic mice expressing human poliovirus receptor.

	Proliferation response in different immunisation groups (mean cpm values)		
Virus antigen	PBS (N=5)	IPV (N=5)	Sabin (N=5)
Coxsackievirus B3	1444	3669	6485
Poliovirus type 1	1927	4898	6738

Grading of coxsackievirus B3 induced pancreatitis and myocarditis as well as the detection of viremia was done two weeks after the coxsackievirus B3 challenge. The presence of viremia was analysed at the same time by detecting viral RNA in serum using a sensitive RT-PCR method. The results are shown in Table 3.

Table 3. Effect of previous poliovirus immunisation on the pathogenesis of subsequent coxsackievirus B3 infection in transgenic mice expressing human poliovirus receptor.

	Immunisation group		
	PBS (N=5)	IPV (N=5)	Sabin (N=5)
Pancreatitis + + +	2	0	4
+ +	3	2	1
+	0	1	0
-	0	2	0
Myocarditis + + +	0	0	0
+ +	0	0	1
+	3	2	3
-	2	3	1
Viremia +	2	3	1
-	3	2	4

In the experiments live poliovirus vaccine (Sabin strain) increased *in vitro* T-cell proliferation responses during subsequent coxsackievirus B3 infection. This increase was observed in proliferation responses against both purified coxsackievirus B3 and poliovirus type 1 (Table 2). This suggests that previous live poliovirus vaccination can augment cellular immune responses during subsequent non-polio enterovirus infection. Previous IPV vaccination also enhanced T-cell responses during subsequent coxsackievirus B3 infection but the effect was weaker that that of live vaccine (Table 2).

Previous immunisation with live poliovirus vaccine (poliovirus type 1, Sabin vaccine strain) increased T-cell infiltration in the pancreas during

subsequent infection with a pancreas-tropic strain of coxsackievirus B3 (Table 3). In contrast, previous IPV vaccination was associated with weak T-cell infiltration in the pancreas as compared to that observed after live poliovirus vaccine or that in control mice. These results suggest that live poliovirus vaccination augment *in vivo* T-cell responses during subsequent non-polio enterovirus infections while killed poliovirus vaccine may have an opposite effect.

There was also a tendency of a low frequency of viremia in mice previously immunised with live poliovirus. This suggests that live poliovirus vaccination facilitates the eradication of subsequent non-polio enterovirus infections.

In another experiment it was found that altogether 9 (92%) out of the twelve poliovaccinated mice had T-cell infiltration in the heart compared to 7 (53%) of the fifteen unvaccinated mice. This suggests that prior challenge by live poliovirus exaggerates T-cell response during CBV3 infection *in vivo*.

Example 2

We have produced and tested formalin-inactivated coxsackievirus B vaccines in mice. These vaccines were produced by inactivating sucrose gradient purified viruses by 14 days incubation at +37 °C in 0.01% formalin in PBS.

Mean IgG1 antibody levels against purified coxsackievirus B3 were determined in Balb/c mice immunized by 3 repeated intramuscular injections with formalin-inactivated coxsackievirus B3 vaccine or phosphate buffered saline (PBS). Injections were given with two weeks intervals (first one at 8 weeks of age) and antibodies were measured at 2 weeks after the last vaccination. Antibody levels are expressed as OD_{492} values in EIA (Table 4).

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Table 4. Antibody levels induced by inactivated coxsackievirus B3 vaccine in mice

	Immunization group	
Serum dilution	PBS (N=5)	Coxsackievirus B3 vaccine (N=5)
1/1600	0.12	0.95
1/6400	0.13	0.47
1/25600	0.14	0.28

Presence of viremia (virus in serum) was determined in BALB/c mice immunized with three repeated intramuscular injections with formalin-inactivated coxsackievirus B3 vaccine or with phosphate buffered saline (PBS) and subsequently infected with a pancreas-tropic strain of coxsackievirus B3 (Nancy strain, 10^6 $TCID_{50}$/mouse). Immunisations were done with two weeks intervals (first one at 8 weeks of age) and mice were infected 2 weeks after the last injection. The presence of virus in serum (viremia) was analysed three days after the infection using the end-point dilution assay of infectivity. End-point dilution of infectivity in LLC-cell cultures is presented in Table 5.

Table 5. Protection against viremia by immunisation with an inactivated coxsackievirus B3 vaccine

	Immunisation group	
Mice	PBS	Coxsackievirus B3 vaccine
1.	10^{-3}	ND
2.	10^{-3}	ND
3.	10^{-1}	ND
4.	10^{-4}	ND
5.	10^{-1}	ND

ND: Not detectable (titre $<10^{-1}$)

As shown in Table 4 immunisation with inactivated coxsackievirus B3 vaccine induced high levels of antibodies as measured against purified coxsackievirus B3 in EIA test. We also found that vaccination completely protected the mice against infection by a pancreas-tropic strain of coxsackievirus B3. Virus could not be detected in the serum in any of the vaccinated animals while all control mice were positive for the virus (Table 5). This vaccine also protected the mice from virus-induced pancreatitis: None of the vaccinated animals had T-cell infiltration in the pancreas while all control mice had a very strong inflammatory response.

These results suggest that inactivated non-polio enterovirus vaccines are effective in the protection against non-polio enterovirus infections. This protection is probably mediated by neutralizing antibodies induced by the vaccine.

Example 3

SJL/J mice were first immunised either with formalin-inactivated poliovirus vaccine (IPV; 0.1 μg/mouse), or with saline (PBS). After 14 days the mice were infected with coxsackievirus B3 intramuscularly (10^6

$TCID_{50}$/mouse). Histopathology of the pancreas was analysed 14 days after the infection. The results are shown in Table 6.

Table 6. Inflammation reaction (T-cell infiltration) in the pancreas of SJL/J mice infected intramuscularly with a pancreas tropic strain of coxsackievirus B3 (Nancy strain).

	Vaccine	
Pancreatic inflammation	PBS (N=5)	IPV (N=5)
Strong	1	4
Moderate	2	1
Not detected	2	0

Our observations indicate that IPV increases the severity of non-polio enterovirus infections. We have found that mice, which have first been immunized by IPV and later infected with a non-polio enterovirus, namely a pancreas tropic Nancy strain of coxsackievirus B3, had more severe pancreatitis than mice which had not previously been immunised with IPV (Table 6).

Mean IgG1 antibody levels against purified coxsackievirus B3 were determined in BALB/c mice immunized with three intramuscular injections with formalin-inactivated poliovirus vaccine (IPV; 0.1 μg per mouse) or with phosphate buffered saline (PBS) and subsequently infected with a pancreas-tropic strain of coxsackivirus B3 (Nancy strain, 10^6 $TCID_{50}$/mouse). Immunisations were done with two weeks intervals (first one at 8 weeks of age), mice were infected 2 weeks after the last injection and antibodies were measured 2 weeks after the infection. Antibody levels are expressed as mean OD_{492} values in EIA (Table 7). IPV was the commercially available poliovirus vaccine Novum purchased by National Public Health Institute of Holland.

Table 7. Effect of immunisation with inactivated poliovirus vaccine on antibody response during subsequent coxsackievirus B3 infection

	Immunisation group	
Serum dilution	PBS (N=5)	IPV (N=5)
1/1600	0.50	0.37
1/6400	0.36	0.11
1/25600	0.32	0.08

IPV vaccination was associated with abnormally low antibody response during subsequent coxsackievirus B3 infection in BALB/c mice (Table 7). This suggests that immunisation with killed poliovirus vaccine may weaken antibody responses during subsequent non-polio enterovirus infections *in vivo*. This, in turn, may increase the severity of non-polio enterovirus infections.

We assume that the harmful effect of IPV is due to its ability to induce Th2-type immune responses. It has been shown previously that inactivated vaccines induce mainly Th2 type responses and that this kind of Th2 bias may increase the severity of natural infections (like in the case of inactivated respiratory syncytial virus and measles vaccine). This harmful effect is manifested particularly in infections caused by other serotypes than that used in the vaccine while infections by the same serotype as that used in the vaccine are totally protected by the vaccine (as shown in our mice experiments described in Table 5). This serotype-specific protection is based on vaccine-induced neutralizing antibodies. Thus IPV vaccination in childhood primes poliovirus specific immune response towards Th2 direction, which imprints T-cell memory in later enterovirus infections. Due to cross-reactive T-cells this Th2-bias will spread to immune responses against non-polio

enteroviruses thus increasing the severity of non-polio enterovirus infections and the risk of their complications like Type 1 diabetes.

In contrast to the harmful effect of IPV on immune protection against non-polio enterovirus infections, OPV has a beneficial effect. As a live vaccine OPV induces stronger T-cell responses than IPV. In addition, this immune response is more balanced resembling that observed in natural enterovirus infections including both Th1- and Th2-type immune responses. This response is targeted to both structural and non-structural virus proteins, while IPV induces only response to structural virus proteins. By inducing strong T-cell responses OPV activates also memory T-cells, which can cross-react between polio and non-polio enteroviruses and booster both T-cell and antibody responses against non-polio enteroviruses. By this mechanism, OPV facilitates the clearance of non-polio enterovirus infections thus preventing from their complications. Thus, the risk of complications of non-polio enterovirus infections (like Type 1 diabetes) can be prevented by OPV.

In addition to the natural non-polio enterovirus infections, OPV can also be used to convert immune responses, which have been induced by inactivated or sub-unit enterovirus vaccines from Th2-type responses to Th1 direction. In this way OPV can be used to protect from the Th2-dependent side effects of inactivated or sub-unit non-polio enterovirus vaccines. This kind of side effects have been described in the context of the use of inactivated respiratory synsytial virus and measles virus vaccines and they include abnormal course of infections, increased severity of the infection, increased risk of complications of the infection and possible development of allergies and asthma.

Accordingly, OPV can be used to dictate the immune response induced by inactivated or subunit enterovirus vaccines to Th1-type responses thus protecting against the side-effects of such vaccines. In contrast, IPV may have an opposite effect increasing the risk of complications of non-polio enterovirus infections by dictating the immune response to Th2 direction.

An additional advantage of OPV over IPV is that as a live virus it induces production of interferon-alpha. It is induced only during virus infections and is the most potent antiviral cytokine (part of the innate immunity). It specifically protects against virus infections and provides protection before the antigen specific immune responses are induced. As a live virus OPV induces interferon-alpha and this induction happens both in mucosal surfaces and

systemically. Vaccine viruses replicate in the gut for several weeks, which means that local production of interferon-alpha persists for prolonged periods in children repeatedly vaccinated by OPV. This will augment to the protective effect of OPV against non-polio enterovirus infections.

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See DM, Tilles JG. Efficacy of a polyvalent inactivated-virus vaccine in protecting mice from infection with clinical strains of group B coxsackieviruses. Scand J Infect Dis 26:739-47, 1994

We claim:

1. Use of oral poliovirus vaccine (OPV) for the manufacture of a vaccine against non-polio enterovirus diseases.

2. Use of oral poliovirus vaccine (OPV) for the manufacture of a vaccine against Type 1 diabetes mellitus (IDDM).

3. Use according to claim 1 or 2 for the manufacture of a vaccine to be administered in repeated doses to children.

4. Use according to claim 3 for the manufacture of a vaccine to be administered by the age of 3 months.

5. Use according to claim 4 for the manufacture of a vaccine to be administered at the age of about 0, 6, 10, and 14 weeks and boosters at older age.

6. Use according to claim 1 or 2 for the manufacture of a vaccine to be administered to pregnant women to protect their offspring against said diseases.

7. Use according to claim 6 for the manufacture of a vaccine to be administered prenatally to the pregnant woman and postnatally to the baby.

8. Use according to any of claims 1 to 7 for the manufacture of a vaccine to be administered in combination with a vaccine, which induces serotype specific immunity against non-polio enteroviruses.

9. Use according to claim 8 wherein said serotype specific immunity inducing vaccine is a killed enterovirus vaccine or a subunit vaccine.

10. Use according to claim 8 wherein said serotype specific immunity inducing vaccine comprises enterovirus antigens representing diabetogenic enterovirus serotypes or a cocktail thereof.

11. Use according to claim 8 wherein said serotype specific immunity inducing vaccine is a vaccine against one or more serotypes selected from the group consisting of coxsackievirus B serotypes 1, 2, 3, 4, 5 and 6, echovirus serotypes 3, 4, 6, 9, 11, 22 and 30, and coxsackievirus A serotypes 9 and 16.

12. A vaccine composition comprising oral poliovirus vaccine (OPV) and a vaccine, which induces serotype specific immunity against non-polio enteroviruses.

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13. The vaccine composition according to claim 12 wherein said serotype specific immunity inducing vaccine is a killed enterovirus vaccine or a subunit vaccine.

14. The vaccine composition according to claim 12 wherein said serotype specific immunity inducing vaccine comprises enterovirus antigens representing diabetogenic enterovirus serotypes or a cocktail thereof.

15. The vaccine composition according to claim 14 wherein said serotype specific immunity inducing vaccine is a vaccine against one or more serotypes selected from the group consisting of coxsackievirus B serotypes 1, 2, 3, 4, 5 and 6, echovirus serotypes 3, 4, 6, 9, 11, 22 and 30, and coxsackievirus A serotypes 9 and 16.

16. Use of a vaccine, which induces serotype specific immunity against one or more serotypes of diabetogenic non-polio enteroviruses selected from the group consisting of coxsackievirus B serotypes 1, 2, 3, 4, 5 and 6, echovirus serotypes 3, 4, 6, 9, 11, 22 and 30, and coxsackievirus A serotypes 9 and 16 for the manufacture of a vaccine against non-polio enterovirus diseases, especially Type 1 diabetes mellitus (IDDM).

17. Use according to claim 16 for the manufacture of a vaccine to be administered to pregnant women or children.

18. Use according to claim 16 for the manufacture of a vaccine to be administered prenatally to the pregnant woman and postnatally to the baby.

19. A method of preventing non-polio enterovirus diseases comprising the administration of an effective amount of oral poliovirus vaccine (OPV) to a human subject.

20. A method of preventing Type 1 diabetes mellitus (IDDM) comprising the administration of an effective amount of oral poliovirus vaccine (OPV) to a human subject.

21. A method of preventing non-polio enterovirus diseases in the offspring comprising the administration of an effective amount of oral poliovirus vaccine (OPV) to pregnant women.

22. A method of preventing Type 1 diabetes mellitus (IDDM) in the offspring comprising the administration of an effective amount of oral poliovirus vaccine (OPV) to pregnant women.

23. A method of preventing non-polio enterovirus diseases, especially IDDM, comprising the administration of repeated doses of an effective amount of oral poliovirus vaccine (OPV) to children.

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24. The method of claim 23 wherein the first OPV is administered by the age of 3 months.

25. The method of claim 24, wherein the OPV is administered at the age of about 0, 6, 10, and 14 weeks and boosters at older age.

26. A method of preventing non-polio enterovirus diseases, especially IDDM, in the offspring comprising the administration of an effective amount of oral poliovirus vaccine (OPV) prenatally to the pregnant woman and postnatally to the baby.

27. The method of any of claims 19 to 26, wherein the administration of OPV is combined with the administration of a vaccine, which induces serotype specific immunity against non-polio enteroviruses.

28. The method of claim 27 wherein the serotype specific immunity inducing vaccine is a killed enterovirus vaccine or a subunit vaccine.

29. The method of claim 27 wherein the serotype specific immunity inducing vaccine comprises enterovirus antigens representing diabetogenic enterovirus serotypes or a cocktail thereof.

30. The method of claim 29 wherein the serotype specific immunity inducing vaccine is a vaccine against one or more serotypes selected from the group consisting of coxsackievirus B serotypes 1, 2, 3, 4, 5 and 6, echovirus serotypes 3, 4, 6, 9, 11, 22 and 30, and coxsackievirus A serotypes 9 and 16.

31. A method of preventing non-polio enterovirus diseases, especially IDDM, comprising administering an effective amount of a vaccine, which induces serotype specific immunity against one or more serotypes of diabetogenic non-polio enteroviruses selected from the group consisting of coxsackievirus B serotypes 1, 2, 3, 4, 5 and 6, echovirus serotypes 3, 4, 6, 9, 11, 22 and 30, and coxsackievirus A serotypes 9 and 16.

32. The method of claim 31 wherein the vaccine is administered to pregnant women or children.

33. The method of claim 31 for preventing the disease in the offspring comprising the administration of the vaccine prenatally to the pregnant woman and postnatally to the baby.

34. A vaccine which induces serotype specific immunity against one or more serotypes of diabetogenic non-polio enteroviruses selected from the group consisting of coxsackievirus B serotypes 1, 2, 3, 4, 5 and 6, echovirus serotypes 3, 4, 6, 9, 11, 22 and 30, and coxsackievirus A serotypes 9 and 16.

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35. A method of avoiding harmful side effects of non-polio enterovirus vaccines, which induce serotype specific immunity against non-polio enteroiruses, said method comprising administering an effective amount of said non-polio enterovirus vaccine simultaneously, before or after administering an effective amount of oral poliovirus vaccine (OPV) to a human subject.

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Abstract

Live virus vaccines comprise attenuated viruses, while other vaccines comprise killed viruses or parts thereof. It has now been found that the immune response induced by oral poliovirus vaccine (OPV), which is a live vaccine, is cross-reactive with non-polio enteroviruses. OPV is therefore useful in the prevention of non-polio enterovirus diseases, especially Type 1 diabetes mellitus (IDDM). OPV is also useful in combination with killed/subunit non-polio enterovirus vaccines, whereby it prevents harmful side-effects of the killed/subunit vaccine by shifting the immune response from a harmful Th2- type response to a Th1 type response.

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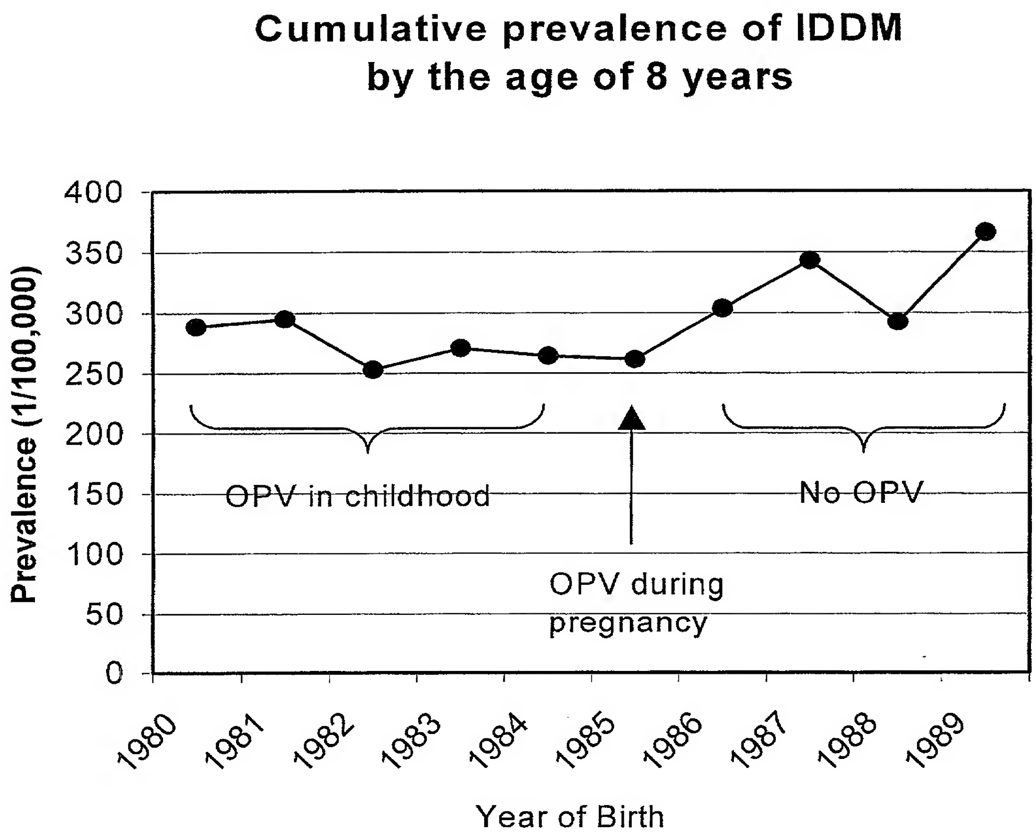
Cumulative prevalence of IDDM
by the age of 8 years
Prevalence (1/100,000)
400
350
300
250
200
150
100
50
0
OPV in childhood
No OPV
OPV during
pregnancy
1980
1981
1982
1983
1984
1985
1986
1987
1988
1989
Year of Birth

Figure 1

#7

Practitioner's Docket No. _____ *PATENT*

Optional Customer No. Bar Code

00140
PATENT TRADEMARK OFFICE

COMBINED DECLARATION AND POWER OF ATTORNEY

(ORIGINAL, DESIGN, NATIONAL STAGE OF PCT, SUPPLEMENTAL, DIVISIONAL, CONTINUATION, OR C-I-P)

As a below named inventor, I hereby declare that:

TYPE OF DECLARATION

This declaration is of the following type:

(check one applicable item below)

[ ] original.
[ ] design.

NOTE: With the exception of a supplemental oath or declaration submitted in a reissue, a supplemental oath or declaration is not treated as an amendment under 37 CFR 1.312 (Amendments after allowance). M.P.E.P. Section 714.16, 7th Ed.

[ ] supplemental.

NOTE: If the declaration is for an International Application being filed as a divisional, continuation or continuation-in-part application, do not check next item; check appropriate one of last three items.

[X] national stage of PCT.

NOTE: If one of the following 3 items apply, then complete and also attach ADDED PAGES FOR DIVISIONAL, CONTINUATION OR C-I-P.

NOTE: See 37 C.F.R. Section 1.63(d) (continued prosecution application) for use of a prior nonprovisional application declaration in the continuation or divisional application being filed on behalf of the same or fewer of the inventors named in the prior application.

[ ] divisional.
[ ] continuation.

NOTE: Where an application discloses and claims subject matter not disclosed in the prior application, or a continuation or divisional application names an inventor not named in the prior application, a continuation-in-part application must be filed under 37 C.F.R. Section 1.53(b) (application filing requirements-nonprovisional application).

[ ] continuation-in-part (C-I-P).

(c) [X] was described and claimed in PCT International Application No. PCT/FI00/00220 filed on 17 March 2000 and as amended under PCT Article 19 on _____ *(if any)*.

SUPPLEMENTAL DECLARATION (37 C.F.R. Section 1.67(b))

(complete the following where a supplemental declaration is being submitted)

[ ] I hereby declare that the subject matter of the

[ ] attached amendment
[ ] amendment filed on _____.

was part of my/our invention and was invented before the filing date of the original application, above identified, for such invention.

ACKNOWLEDGMENT OF REVIEW OF PAPERS AND DUTY OF CANDOR

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information, which is material to patentability as defined in 37, Code of Federal Regulations, Section 1.56,

(also check the following items, if desired)

[ ] and which is material to the examination of this application, namely, information where there is a substantial likelihood that a reasonable Examiner would consider it important in deciding whether to allow the application to issue as a patent, and

[ ] in compliance with this duty, there is attached an information disclosure statement, in accordance with 37 C.F.R. Section 1.98.

PRIORITY CLAIM (35 U.S.C. Section 119(a)-(d))

NOTE: "The claim to priority need be in no special form and may be made by the attorney or agent if the foreign application is referred to in the oath or declaration as required by Section 1.63. The claim for priority and the certified copy of the foreign application specified in 35 U.S.C. Section 119(b) must be filed in the case of an interference (Section 1.630), when necessary to overcome the date of a reference relied upon by the examiner, when specifically required by the examiner, and in all other situations, before the patent is granted. If the claim for priority or the certified copy of the foreign application is filed after the date the issue fee is paid, it must be accompanied by a petition requesting entry and by the fee set forth in Section 1.17(i). If the certified copy is not in the English language, a translation need not be filed except in the case of interference; or when necessary to overcome the date of a reference relied upon by the examiner; or when specifically required by the examiner, in which event an English language translation must be filed together with a statement that the translation of the certified copy is accurate." 37 C.F.R. Section 1.55(a).

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed.

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(complete (d) or (e))

(d) [ ] no such applications have been filed.
(e) [X] such applications have been filed as follows.

NOTE: Where item (c) is entered above and the International Application which designated the U.S. itself claimed priority check item (e), enter the details below and make the priority claim.

PRIOR FOREIGN/PCT APPLICATION(S) FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS APPLICATION AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. SECTION 119(a)-(d)

COUNTRY (OR INDICATE IF PCT)	APPLICATION NUMBER	DATE OF FILING DAY, **MONTH**, YEAR	PRIORITY CLAIMED UNDER 35 USC 119
USA	60/140,872	24 June 1999	[X]YES [ ]NO
			[ ]YES [ ]NO
			[ ]YES [ ]NO
			[ ]YES [ ]NO
			[ ]YES [ ]NO

CLAIM FOR BENEFIT OF PRIOR U.S. PROVISIONAL APPLICATION(S)
(35 U.S.C. Section 119(e))

I hereby claim the benefit under Title 35, United States Code, Section 119(e) of any United States provisional application(s) listed below:

PROVISIONAL APPLICATION NUMBER	**FILING DATE**
/ _____	_____
/ _____	_____
/ _____	_____

CLAIM FOR BENEFIT OF EARLIER U.S./PCT APPLICATION(S) UNDER 35 U.S.C. SECTION 120

[ ] The claim for the benefit of any such applications are set forth in the attached ADDED PAGES TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR DIVISIONAL, CONTINUATION OR CONTINUATION-IN-PART (C-I-P) APPLICATION.

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ALL FOREIGN APPLICATION(S), *IF ANY,* FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

NOTE: If the application filed more than 12 months from the filing date of this application is a PCT filing forming the basis for this application entering the United States as (1) the national stage, or (2) a continuation, divisional, or continuation-in-part, then also complete ADDED PAGES TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR DIVISIONAL, CONTINUATION OR C-I-P APPLICATION for benefit of the prior U.S. or PCT application(s) under 35 U.S.C. Section 120.

POWER OF ATTORNEY

I hereby appoint the following practitioner(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

(list name and registration number)

JOSEPH H. HANDELMAN, 26179

JOHN RICHARDS, 31053

RICHARD J. STREIT, 25765

PETER D. GALLOWAY, 27885

IAIN C. BAILLIE, 24090

RICHARD P. BERG, 28145

JULIAN H. COHEN, 20302

WILLIAM R. EVANS 25858

JANET I. CORD, 33778

CLIFFORD J. MASS, 30086

CYNTHIA R. MILLER, 34678

(Check the following item, if applicable)

[ ] I hereby appoint the practitioner(s) associated with the Customer Number provided below to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

[ ] Attached, as part of this declaration and power of attorney, is the authorization of the above-named practitioner(s) to accept and follow instructions from my representative(s).

NOTE: "Special care should be taken in continuation or divisional applications to ensure that any change of correspondence address in a prior application is reflected in the continuation or divisional application. For example, where a copy of the oath or declaration from the prior application is submitted for a continuation or divisional application filed under 37 CFR 1.53(b) and the copy of the oath or declaration from the prior application designates an old correspondence address, the Office may not recognize, in the continuation or divisional application, the change of correspondence address made during the prosecution of the prior application. Applicant is required to identify the change of correspondence address in the continuation or divisional application to ensure that communications from the Office are mailed to the current correspondence address. 37 CFR 1.63(d)(4)." Section 601.03, M.P.E.P., 7th Ed

SEND CORRESPONDENCE TO

Ladas & Parry
26 West 61^st Street
New York, N.Y. 10023

DIRECT TELEPHONE CALLS TO:
(Name and telephone number)

(complete the following if applicable)

Since this filing is a [ ] continuation [ ] divisional there is attached hereto a Change of Correspondence Address so that there will be no question as to where the PTO should direct all correspondence.

DECLARATION

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

INVENTORSHIP IDENTIFICATION

WARNING: *If the inventors are each not the inventors of all the claims, an explanation of the facts, including the ownership of all the claims at the time the last claimed invention was made, should be submitted.*

My residence, post office address and citizenship are as stated below, next to my name. I believe that I am the original, first and sole inventor *(if only one name is listed below)* or an original, first and joint inventor *(if plural names are listed below)* of the subject matter that is claimed, and for which a patent is sought on the invention entitled:

TITLE OF INVENTION

Prevention of type 1 diabetes and other non-polio enterovirus diseases

---

SPECIFICATION IDENTIFICATION

The specification of which:

(complete (a), (b), or (c))

(a) [ ] is attached hereto.

NOTE: "The following combinations of information supplied in an oath or declaration filed on the application filing date with a specification are acceptable as minimums for identifying a specification and compliance with any one of the items below will be accepted as complying with the identification requirement of 37 C.F.R. Section 1.63:

"(1) name of inventor(s), and reference to an attached specification which is both attached to the oath or declaration at the time of execution and submitted with the oath or declaration on filing;

"(2) name of inventor(s), and attorney docket number which was on the specification as filed; or

"(3) name of inventor(s), and title which was on the specification as filed."

Notice of July 13, 1995 (1177 O.G. 60).

(b) [ ] was filed on _____, [ ] as Application No. _____
[ ] and was amended on _____ (if applicable).

NOTE: Amendments filed after the original papers are deposited with the PTO that contain new matter are not accorded a filing date by being referred to in the declaration. Accordingly, the amendments involved are those filed with the application papers or, in the case of a supplemental declaration, are those amendments claiming matter not encompassed in the original statement of invention or claims. See 37 C.F.R. Section 1.67.

NOTE: "The following combinations of information supplied in an oath or declaration filed after the filing date are acceptable as minimums for identifying a specification and compliance with any one of the items below will be accepted as complying with the identification requirement of 37 C.F.R. Section 1.63:

(A) application number (consisting of the series code and the serial number, e.g., 08/123,456);
(B) serial number and filing date;
(C) attorney docket number which was on the specification as filed;
(D) title which was on the specification as filed and reference to an attached specification which is both attached to the oath or declaration at the time of execution and submitted with the oath or declaration; or
(E) title which was on the specification as filed and accompanied by a cover letter accurately identifying the application for which it was intended by either the application number (consisting of the series code and the serial number, e.g., 08/123,456), or serial number and filing date. Absent any statement(s) to the contrary, it will be presumed that the application filed in the PTO is the application which the inventor(s) executed by signing the oath or declaration.

M.P.E.P. Section 601.01(a), 7th ed.

(check proper box(es) for any of the following added page(s) that form a part of this declaration)

[ ] **Signature** for fourth and subsequent joint inventors. *Number of pages added* _____

* * *

[ ] **Signature** by administrator(trix), executor(trix) or legal representative for deceased or incapacitated inventor. *Number of pages added* _____

* * *

[ ] **Signature** for inventor who refuses to sign or cannot be reached by person authorized under 37 C.F.R. Section 1.47. *Number of pages added* _____

* * *

[ ] Added page for **signature** by one joint inventor on behalf of deceased inventor(s) where legal representative cannot be appointed in time. (37 C.F.R. Section 1.47)

* * *

[ ] Added pages to combined declaration and power of attorney for divisional, continuation, or continuation-in-part (C-I-P) application.

[ ] Number of pages added _____

* * *

[ ] Authorization of practitioner(s) to accept and follow instructions from representative.

(If no further pages form a part of this Declaration, then end this Declaration with this page and check the following item)

[X] This declaration ends with this page.

SIGNATURE(S)

NOTE: Carefully indicate the family (or last) name, as it should appear on the filing receipt and all other document.

NOTE: Each inventor must be identified by full name, including the family name, and at least one given name without abbreviation together with any other given name or initial, and by his/her residence, post office address and country of citizenship. 37 C.F.R. Section 1.63(a)(3).

NOTE: Inventors may execute separate declarations/oaths provided each declaration/oath sets forth all the inventors. Section 1.63(a)(3) requires that a declaration/oath, inter alia, identify each inventor and prohibits the execution of separate declarations/oaths which each sets forth only the name of the executing inventor. 62 Fed. Reg. 53,131, 53,142, October 10, 1997,

1-00

Full name of sole or first inventor
Heikki HYÖTY
(Given Name) *(Middle Initial or Name)* *Family (Or Last Name)*

Inventor's signature _____

Date 12/12/2001 **Country of Citizenship** Finland

Residence MINNA CANTHIN KATU 3 B

Post Office Address FIN-33230 TAMPERE, FINLAND FIX

,,,,,,

2-00

Full name of second joint inventor, if any
Mikael KNIP
(Given Name) *(Middle Initial or Name)* *Family (Or Last Name)*

Inventor's signature _____

Date 12/12/2001 **Country of Citizenship** Finland

Residence Palomäentie 11 a 2

Post Office Address FIN-33230 Tampere, Finland FIX

,,,,,,

Full name of third joint inventor, if any

(Given Name) *(Middle Initial or Name)* *Family (Or Last Name)*

Inventor's signature _____

Date _____ **Country of Citizenship** _____

Residence _____

Post Office Address _____